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An Analysis of the Microbial Quality of Packaged Water in Four Sites in Latin America

Karla Feeser

An Analysis of the Microbial Quality of Packaged Water in Four Sites in Latin America

By

Karla Feeser

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By

Karla Feeser

Approved:

Committee Chair

Committee Member

Date

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Abstract

Karla Feeser

An Analysis of the Microbial Quality of Packaged Water in Four sites in Latin America

(Thesis Chair: Dr. Christine Stauber, GSU School of Public Health)

Much of the death and disease caused by diarrhea in low and middle income countries could be alleviated with better access to safe drinking water; yet, globally 780 million people lack access. Private, small-scale packaged water providers can and do play an important role in meeting the water needs of populations in impoverished or developing countries where public centralized water utilities are not feasible or not trusted; however, recent studies have indicated concerns about the quality and safety of packaged water. This pilot study seeks to identify factors that may be associated with packaged water quality in four sites in Central and South America. It concludes that a large portion of the contamination found in water that is packaged in reusable containers may stem from inadequate disinfection of the containers between uses, and recommends further research on simple, effective disinfection protocols that are practical for use in low-resource settings. Finally, packaged water enterprises should be considered by policymakers who regulate drinking water quality.

KEY WORDS: packaged water, drinking water quality, SWE

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The author of this thesis is:

Karla Feeser
834 Argonne Ave NE, #2
Atlanta, GA 30308

The Chair of the committee for this thesis is:
Christine E. Stauber, PhD
Institute of Public Health

Georgia State University
P.O. Box 3995
Atlanta, GA 30302-3995

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Karla Feeser

Curriculum Vitae

Home Address: 834 Argonne Ave NE, #2
Atlanta, GA 30308
Phone: 443 – 624 -0903

Education:

2010-2012 B.S. Towson University, Baltimore, MD
2008-2010 A.A. Community College of Baltimore County

Professional Experience:

2015- Present Graduate Research Assistant
Georgia State University

2015-Present Surveillance Officer
Georgia Emerging Infections Program

2015-2015 Water Research Fellow
Water Ecuador 501c3

2012-2015 Microbiologist
Division of Parasitic Diseases and Malaria
Centers for Disease Control and Prevention

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Chapter I: Introduction

Much of the death and disease caused by diarrhea in low and middle income countries could be alleviated with better access to safe drinking water; yet, globally 780 million people lack access (World Health Organization (WHO) & UNICEF, 2012; WHO, 2014; Prüss-Ustün et al., 2014). In developing regions, piped drinking water supplies reach only 46% of the population, due, in part, to disparities in access to safe water infrastructure. Piped water systems often do not reach lower income or rural areas in low-middle income countries where the established infrastructure required of a centralized water utility is often missing, and the high cost to develop missing infrastructure may be prohibitive (Dada, 2011). Even where a centralized utility is available, it may be perceived as unsafe to drink (Hatt, 2006).

In Latin America, access to improved drinking water sources and piped water supplies in particular has risen substantially since 1990 to reach approximately 94% coverage, although substantial disparities between urban and rural areas still exist (WHO & UNICEF, 2012). However, throughout the region, there is mistrust in the quality of the water provided through centralized utilities (de Queiroz, Doria, 2013; Espinosa-García et al., 2015; Jain, 2014).). Indeed, the microbial quality of piped drinking water sources is not globally monitored; they are thought to be clean due to the nature of their construction. If these sources are poorly maintained or constructed, they may be contaminated with fecal pathogens. Thus, it is likely that the number of people with access to a safe and improved source of drinking water is even less than estimates allow (WHO & UNICEF, 2012).

Private, small-scale packaged water providers can and do play an important role in meeting the water needs of populations in impoverished or developing countries where public centralized water utilities are not feasible (Solo, 1999). Packaged water, intended to be potable, is sold in a variety of vessels—sachets, pouches, boxes, cans, and reusable and disposable bottles—and typically vended through both water distributors and water kiosks (Dada, 2011). The number of people relying on packaged water, particularly in large twenty liter reusable bottles, is on the rise, even among those households with a connection to a public water utility (JMP, 2011). Oftentimes, even the lowest-income families tolerate the expense because they perceive the water as safe to drink, more so than other sources (Kjellen, 2006).

Packaged water is significantly less likely to be contaminated than other water sources, including improved water sources such as piped water (Williams et al., 2015). However, substantial heterogeneity is found seen across study sites—with more than 40% of studies reporting packaged water to be of equal or lesser quality than piped water. Additionally, significant disparities exist between low income countries (LICs) and upper-middle and high income countries (UM/HICs) in contamination levels of packaged water. Poorer countries may face more obstacles in the monitoring and regulation of packaged water enterprises. In fact, even as small water enterprises gain recognition as viable drinking water alternative, in many developing nations SWEs operate either completely unregulated or unregistered (Dada, 2011). Consequently, studies investigating the microbial contamination of packaged water often find that the quality of packaged water sold by SWE meets neither international guidelines nor national standards, when they are in place (Fisher et al., 2015; Halage et al., 2015).

For packaged water sold in reusable bottles, inadequate cleaning methods may serve as a potential source of contamination before the point of sale or use (Falcone-Dias, 2012; Marzano, 2011). However, despite the existence of international and national drinking water guidelines, there is no available evidence to suggest that these kinds of stringent processes for the disinfection of reusable containers are enforced in many low income countries throughout the world.

This study seeks to characterize the quality of packaged water available for purchase in four sites in Central and South America: La Paz, Bolivia; Tegucigalpa, Honduras; Muisne, Ecuador; and Tena, Ecuador. Furthermore, it identifies factors that may be associated with packaged water quality, particularly in reusable containers, and discusses recommendations for the regulation of distributors that provided packaged water to underserved communities around the world.

1.1 Purpose of Research

As the activity and importance of SWEs and packaged water vendors increase, more research is needed to assess the quality of water sold, and to inform policies that regulate the private water sector throughout the world. This study seeks to identify factors that may contribute to the deterioration of packaged water quality, and to establish recommendations for business owners and managers of SWEs that provided packaged water to underserved communities around the world.

Chapter 2: Literature Review

2.1 Background

Diarrheal disease contributes to an estimated 1.5 million deaths each year, including 760,000 deaths among children under the age of five ("WHO | Diarrhoeal disease," n.d.). Globally, these diseases represent the second leading cause of childhood mortality (Liu et al., 2012). In Latin American countries, twenty to thirty percent of children under five experience diarrhea each month, with the burden falling heaviest on those of lower socioeconomic status (Hatt & Waters, 2006).

Bacteria, viruses or parasites transmitted via the fecal-oral route are the most common causes of diarrhea, especially in the developing world. Fecal-oral transmission occurs through contact with food, water or surfaces that have been contaminated with fecal matter from an infected individual (Kelly, 2011).Important pathogens include norovirus and rotavirus, which are highly infectious and easily transmitted. *Shigella* and toxigenic *E. coli* are common bacterial agents of serious diarrhea, and *Cryptosporidium* exemplifies a potentially culpable parasite (Kotloff et al., 2013).

Furthermore, diarrheal diseases impact the absorption of nutrients in the gut, making them a leading cause of malnutrition worldwide. Malnutrition is a complex health outcome of diarrhea because it leads to an increase in both the frequency and duration of diarrheal episodes. This positive feedback loop of malnutrition and diarrhea not only increases childhood mortality, but also leads to stunted growth, lowered physical fitness, and impaired cognition and school performance (Gracey, 1996). The resulting impact on education, especially among young girls, often manifests into decreased productivity and limited economic opportunity and growth (Jasper, 2012), which bears lifelong economic implications.

It is estimated that 58% of deaths due to diarrhea can be explained by poor water, sanitation and hygiene (WHO, 2014). Of those, approximately 500,000 are attributable to inadequate drinking water (Prüss-Ustün et al., 2014). Thus, much of the death and disease caused by diarrhea in developing nations could be alleviated with better access to safe drinking water.

2.2 Access to Healthy Water

The Millennium Development Goals (MDG) set forth by the United Nations in 2000 embody a commitment by world leaders to end extreme poverty (UN, 2015). The seventh goal—to ensure environmental sustainability—includes Target 7.C, which aims to "halve, by 2015, the proportion of people without sustainable access to safe drinking water and basic sanitation" (UN, 2015). Progress toward achieving this target is monitored by a collaborative group formed by UNICEF and the World Health Organization named the Joint Monitoring Programme (JMP) for Water Supply and Sanitation (WHO/UNICEF, 2010). JMP plays a key role in the development of measurable indicators used to evaluate progress toward the fulfillment of water and sanitation targets. JMP breaks drinking-water sources down into two main categories: improved sources and unimproved sources. To be classified as improved, a drinking- water source must "by nature of its construction and when properly used, adequately protect the source from outside contamination, particularly faecal matter" (WHO/UNICEF, n.d.). For example, improved water sources can include water that is piped directly to the home or to a public tap, tubewells that draw from groundwater supplies, rainwater, or wells that are dug, constructed and covered to prevent contamination from entering the source water. However, in practice, these definitions do not stipulate that improved water sources be free of microbial contamination in order to be counted toward progress in achieving Target 7.C, thus recent estimates of the number of people with access to safe drinking water may be inflated (Bain et al., 2014).

2.3 The Role of Packaged Water and Small Water Enterprises

Though one may expect packaged and bottled water to be generally safe for consumption, they are not considered improved drinking-water sources by the JMP (WHO/UNICEF, n.d.). Bottled water is not sufficient, affordable or feasible for use as a sole household water source. For basic uses, including drinking, personal hygiene and cooking, the average person needs approximately 50 liters of water per day (Gleick, 1996). For this reason, bottled water is only considered to be an improved drinking-water source if the household uses some other improved water source for other tasks (WHO/UNICEF, 2011). Nevertheless, packaged and bottled drinking water can fill gaps in areas where improved water sources are unavailable or mistrusted (Hatt & Waters, 2006). Private, small-scale providers can and do play an important role in meeting the water needs of populations in impoverished or developing countries where public centralized water utilities are not feasible (Solo, 1999). Historically, these private water providers have been most abundant in areas where piped water was unsafe, unreliable or economically impossible (Zaroff, 1984). Many developing countries lack the established infrastructure required of a centralized water utility, and the high cost to develop missing infrastructure is often prohibitive (Dada, 2011). Small water enterprises (SWEs) have proven to be efficient and sustainable as alternative drinking water providers in low-resources settings (Bhatt, 2014).

Water vendors, or SWEs can take on many forms. Privately owned connection networks pull water from the public utility and pipe it directly to homes or community standpipes. Water distributors deliver water to households in a variety of ways, from large tankers, which truck water in and dispense into household containers, to packaged water vendor who deliver water bottled in reusable containers. Water kiosks provide a stationary point of sale where individuals can purchase water to carry home by re-filling household containers, purchasing or exchanging empty containers for full containers, or purchasing water in disposable containers such as sachets or small bottles (Kjellen, 2006). Packaged water, intended to be potable, is sold in a variety of vessels—sachets, pouches, boxes, cans, and reusable and disposable bottles—and typically vended through both water distributors and water kiosks (Dada, 2011).

The number of people relying on packaged water, particularly in large twenty liter reusable bottles, is on the rise, even among those households with a connection to a public water utility (JMP, 2011). Oftentimes, even the lowest-income families tolerate the expense because they perceive the water as safe to drink, more so than other sources (Kjellen, 2006).

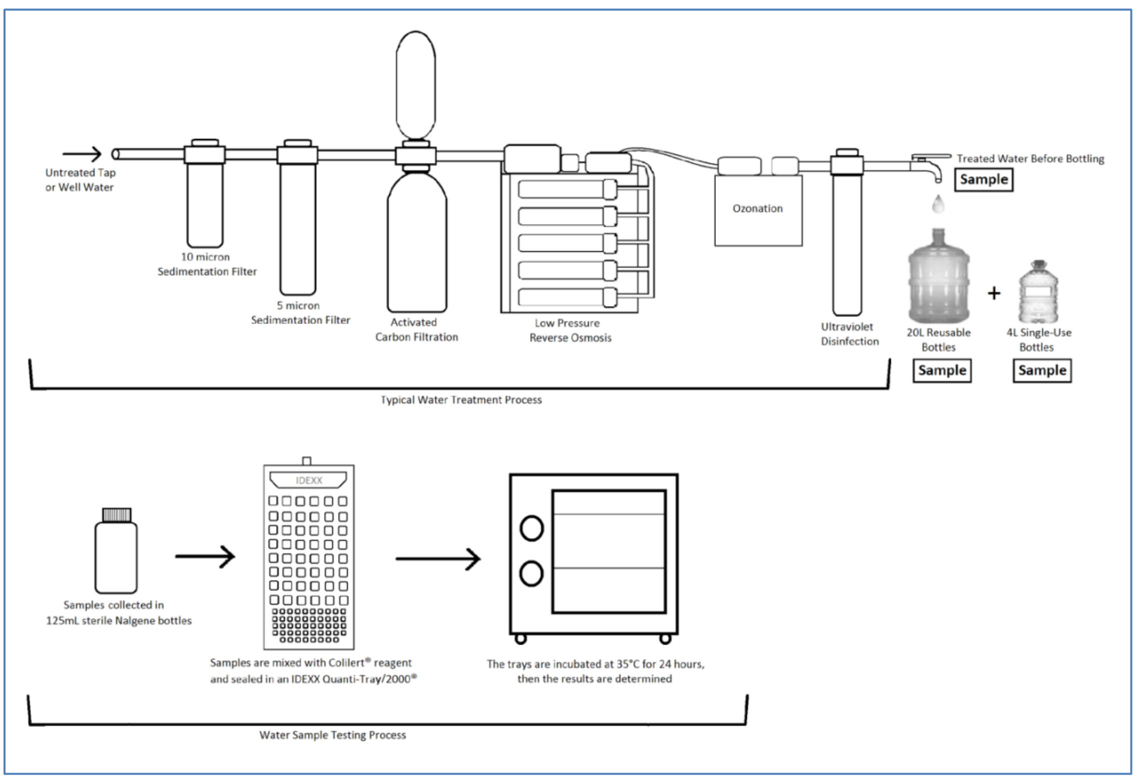
2.3.1 Packaged Water Quality

A 2015 systematic review and meta-analysis found that, overall, packaged water is significantly less likely to be contaminated than other water sources, including improved water sources such as piped water (Williams et al., 2015). However, substantial heterogeneity was seen across study sites—with more than 40% studies reporting that packaged water was of equal or lesser quality than piped water. Additionally, the analysis revealed significant disparities between low income countries (LICs) and upper-middle and high income countries (UM/HICs) in contamination levels of packaged water, even when accounting for more prevalent use of sachet water—which is more likely to be contaminated than bottled water—among LICs. Poorer countries may face more obstacles in the monitoring and regulation of packaged water enterprises. In fact, even as SWEs gain recognition as viable drinking water alternative, in many developing nations SWEs operate either completely unregulated or unregistered (Dada, 2011). Consequently, studies investigating the microbial contamination of packaged water often find that the quality of packaged water sold by SWE meets neither international guidelines nor national standards, when they are in place (Fisher et al., 2015; Halage et al., 2015).

Packaged drinking water can be contaminated at any point along the supply line—from source to household. First, water is not necessarily treated before being packaged and sold and therefore is sometimes only as safe as the source water. Typically, packaged water sold as mineral water is untreated, but assumed safe for consumption because it is pulled from underground springs and bottled on-site (Falcone-Dias, 2012). Non-mineral water, or water from other sources, is most often treated prior to bottling or packaging to improve its quality. WHO recommends that multiple disinfection strategies be used in the treatment of drinking water to ensure an adequate reduction in pathogens in the event that the source water is highly contaminated or contaminated with resistance microorganisms, such as *Cryptosporidium*, which is not susceptible to chlorination alone (WHO, 2011). Thus, a combination of chemical disinfection and filtration is often used.

A typical treatment system for packaged water is shown in Figure 1. Filtration of water can occur through many types of media, and its efficacy depends largely on pore size. For example, cloth filters are useful for removing large microbes (> 20 µm), such as parasitic worms or microbes that are associated with copepods. Filters made from ceramic or carbon have smaller pores and are able to remove more organisms, such as small protozoa, and viruses. Granular media filtration, such as sand filtration, can filter out organisms based on the coarseness or grain of the sand particles and the rate of filtration. The most popular chemical disinfectants are chlorine and ozone gas. Chlorine is often added so that a residual concentration of the chemical remains in water (Impellitteri, 2007). Ozone is used to disinfect water, but no residual concentration remains. The use of chemical disinfectants can lead to the creation of disinfection by products in drinking water. UV radiation of water can be achieved through solar disinfection technology or through the use of UV lamps. Processes to improve the taste, odor or appearance of water may also be included. For example, demineralization of water—water softening—can be achieved through the use of a cation exchange resin (World Health Organization, 2011).

Figure 1. Schematic of a typical water treatment plant and testing protocol.



Purified water can become contaminated upon introduction into an unclean container, such as a reusable bottle that has not been appropriately disinfected. Additionally, purified water, even stored in a clean container can become contaminated by pathogens introduced through use in the household (Levy, 2008).

Only a handful of studies mention reusable bottles as a potential source of contamination before the point of sale or use (Falcone-Dias, 2012; Marzano, 2011). A study on the microbiological quality of bottled mineral water reported a higher concentration of total coliform bacteria, *E. coli* and *P. aeruginosa* in 20L bottles than in 1.5 L bottles, but did not collect sufficient information to test the hypothesis that the higher contamination was the result of reusable bottles that had not been cleaned appropriately (Falcone-Dias, 2012). Similarly, a 2010 study examining bottled water dispensers as a potential source for contamination in large reusable containers noted the presence of bacteria in the bottles, and suggested that it might be attributable to either the water production plant or the plastic bottles themselves (Marzano, 2011).

When testing the quality of drinking water, the drinking water guidelines set forth by WHO dictate that there should be no detectable *E .coli* per 100mL of water intended for drinking, including packaged or bottled water (WHO, 2011).

Companies that produce and sell bottled water in the United States are regulated by the U.S. Food and Drug Administration (FDA) and have standard protocols for inspecting and cleaning reusable containers(Nestle Waters North American Inc., 2010).   However, despite the existence of international and national drinking water guidelines, there is no available evidence to suggest that these kinds of stringent process for the disinfection of reusable containers are enforced in many low income countries throughout the world.

2.4 Study Sites

In Latin American countries, the use of packaged and bottled drinking water is substantial. In 2008, it is estimated that a projected volume of 5,343 million liters of bottled water were consumed in Latin America (Datamonitor, 2004). In parts of Central and South America, the industry has grown as much of 55% over the last five years (“Informe anual bebidas 2015,” 2015). Although exact data are limited, reports suggest that people throughout Central and South America are increasingly dependent on private water enterprises for purified drinking water (Kjellen, 2006; Pacheco-Vega, 2015).

2.4.1 El Alto, La Paz, Bolivia

In the late 1990s, issues of water scarcity and poor water infrastructure led to a massive privatization of Bolivia’s water resources (Baer, 2015). Particularly, in the city of Cochabamba and in the peri-urban areas surrounding La Paz, concessions that allowed large multi-national corporations to control water utilities were met with backlash and social uprisings. The concessions were reversed, and since then the Bolivian government has amended their constitution to include water as a human right (Baer, 2015)). The Bolivian government ministries have gone through several phases of restructuring as it pertains to water resource provisioning, regulation, and policy making. The structure and governance of the public water utility in the area of La Paz and the surrounding community of El Alto, where the current study was conducted, have been in transition since 2007 (Baer, 2015). Piped water coverage has risen in Bolivia—90 percent of households use piped water or another improved water source compared to just 69 percent in 1990—but there is large disparity between urban and rural areas, where coverage hovers just under 60 percent (WHO/UNICEF, 2015c). Furthermore, reports have indicated that the majority of water provided through the public water utility is untreated and unsafe to drink (Fundacion Abril, 2013). Thus, SWEs still play an important role in the study area. In particular, informal water distributors and water kiosks operate on a largely unregulated basis (Wutich, 2016).

2.4.2 Tegucigalpa, Honduras

According to most recent estimates, 90 percent of households in Honduras used piped water (WHO/UNICEF, 2015b). Nevertheless, up to thirty percent of households in some communities continue to use bottled water for drinking and cooking (Halder, 2013). Generally, the bottled water industry throughout Honduras is substantial, owing chiefly to mistrust in the quality of water and reliability of service provided by municipal systems (Public-Private Infrastructure Advisory Facility, 2003). In Honduras, the Technical Standard for Drinking Water Quality (TSDWQ) regulates the production of bottled water. Like the WHO guidelines, the allowable limit for *E. coli* in drinking water under the TSDWQ is zero CFU per 100mL (Ministerio de Salud, 1995).

2.4.3 Muisne and Tena, Ecuador

The percentage of households using improved water sources n Ecuador has risen significantly since 1990—when only 38 percent of rural households and 59 percent of urban households had access to piped water. However, even today nearly 30 percent of rural households are without access to piped water, and coverage throughout the country has reached only 85 percent (WHO/UNICEF, 2015a). Two of the four present study sites were located in rural Ecuador. The first, Muisne, is a coastal town in the Esmeraldas province in the northern part of the country. In this part of the country, people relying chiefly on untreated water for drinking (Levy, 2012). The second site was located in the interior of the county just east of the Amazon Rainforest's westernmost edge. Tena and the smaller neighboring town of Archidona lie along the River Napo, which also serves as the main drinking water reservoir for the city's municipal water systems.

References

Baer, M. (2015). From Water Wars to Water Rights: Implementing the Human Right to Water in Bolivia. Journal of Human Rights, 14(3), 353–376. http://doi.org/10.1080/14754835.2014.988782

Bain, R., Cronk, R., Wright, J., Yang, H., Slaymaker, T., & Bartram, J. (2014). Fecal Contamination of Drinking-Water in Low- and Middle-Income Countries: A Systematic Review and Meta-Analysis. PLoS Medicine, 11(5). http://doi.org/10.1371/journal.pmed.1001644

Bhatt, J. (2014). Comparison of small-scale providers' and utility performance in urban water supply: the case of Maputo, Mozambique. Water Policy, 16(1), 102–123. http://doi.org/10.2166/wp.2013.083

Dada, A. (2011). Packaged water: optimizing local processes for sustainable water delivery in developing nations. Globalization & Health, 7(1), 24–31. http://doi.org/10.1186/1744-8603- 7-24

Falcone-Dias, M. F., Emerick, G. L., & Farache-Filho, A. (2012). Mineral water: a microbiological approach. Water Science & Technology: Water Supply, 12(5), 556–562. http://doi.org/10.2166/ws.2012028

Fisher, M. B., Williams, A. R., Jalloh, M. F., Saquee, G., Bain, R. E. S., & Bartram, J. K. (2015). Microbiological and Chemical Quality of Packaged Sachet Water and Household Stored Drinking Water in Freetown, Sierra Leone. PLOS ONE, 10(7), e0131772. http://doi.org/10.1371/journal.pone.0131772

Fundacion Abril. (2013, June 6). Agua y Saneamiento. Retrieved from http://www.fundacionabril.org/about-2/agua-y-saneamiento/

Gleick, P. (1996). Basic Water Requirements for Human Activities: Meeting Basic Needs. Water International, 21, 83–92.

Gracey, M. (1996). Diarrhea and Malnutrition: A Challenge for Pediatricians. Journal of Pediatric Gastroenterology & Nutrition, 22(1), 6–16.

Halage, A. A., Ssemugabo, C., Ssemwanga, D. K., Musoke, D., Mugambe, R. K., Guwatudde, D., & Ssempebwa, J. C. (2015). Bacteriological and physical quality of locally packaged drinking water in Kampala, Uganda. Journal of Environmental and Public Health, 2015, 942928. http://doi.org/10.1155/2015/942928

Halder, G. E., Bearman, G., Sanogo, K., & Stevens, M. P. (2013). Water sanitation, access, use and self-reported diarrheal disease in rural Honduras. Rural and Remote Health, 13(2), 2413.

Hatt, L. E., & Waters, H. R. (2006). Determinants of child morbidity in Latin America: A pooled analysis of interactions between parental education and economic status. Social Science & Medicine, 62(2), 375–386. http://doi.org/10.1016/j.socscimed.2005.06.007

Jasper, C., Le, T.-T., & Bartram, J. (2012). Water and sanitation in schools: a systematic review of the health and educational outcomes. International Journal Of Environmental Research And Public Health, 9(8), 2772–2787. http://doi.org/10.3390/ijerph9082772

Kelly, P. (2011). Infectious diarrhoea. Medicine, 39(4), 201–206. http://doi.org/10.1016/j.mpmed.2011.01.008

Kjellen, M., & McGranahan, G. (2006). Informal Water Vendors and the Urban Poor. International Institute for Environment and Development (IIED). Retrieved from http://pubs.iied.org/pdfs/10529IIED.pdf

Kotloff, K. L., Nataro, J. P., Blackwelder, W. C., Nasrin, D., Farag, T. H., Panchalingam, S., … Levine, M. M. (2013). Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. The Lancet, 382(9888), 209–22. http://doi.org/http://dx.doi.org.ezproxy.gsu.edu/10.1016/S0140-6736(13)60844-2

Levy, K., Nelson, K. L., Hubbard, A., & Eisenberg, J. N. S. (2008). Following the Water: A Controlled Study of Drinking Water Storage in Northern Coastal Ecuador. Environmental Health Perspectives, 116(11), 1533–1540. http://doi.org/10.1289/ehp.11296

Levy, K., Nelson, K. L., Hubbard, A., & Eisenberg, J. N. S. (2012). Rethinking Indicators of Microbial Drinking Water Quality for Health Studies in Tropical Developing Countries: Case Study in Northern Coastal Ecuador. The American Journal of Tropical Medicine and Hygiene, 86(3), 499–507. http://doi.org/10.4269/ajtmh.2012.11-0263

Liu, L., Johnson, H. L., Cousens, S., Perin, J., Scott, S., Lawn, J. E., … Child Health Epidemiology Reference Group of WHO and UNICEF. (2012). Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet (London, England), 379(9832), 2151–2161. http://doi.org/10.1016/S0140-6736(12)60560-1

Marzano, M. a., Ripamonti, B., & Balzaretti, C. m. (2011). Short communication: Monitoring the bacteriological quality of Italian bottled spring water from dispensers. Food Control, 22, 333–336. http://doi.org/10.1016/j.foodcont.2010.06.014

Ministerio de Salud. (1995). Norma tecnica para la calidad del Agua Potable. Gobierno de Honduras.

Nestle Waters North American Inc. (2010). Poland Spring Water Quality Report.

Stamford: Nestle Waters.

Prüss-Ustün, A., Bartram, J., Clasen, T., Colford, J. M., Cumming, O., Curtis, V., … Cairncross, S. (2014). Burden of disease from inadequate water, sanitation and hygiene in low- and middle-income settings: a retrospective analysis of data from 145 countries. Tropical Medicine & International Health, 19(8), 894–905. http://doi.org/10.1111/tmi.12329

Public-Private Infrastructure Advisory Facility. (2003). Private Solutions for Infrastructure in Honduras. World Bank Publications.

Solo, T. M. (1999). Small-scale entrepreneurs in the urban water and sanitation market. Environment and Urbanization, 11(1), 117–132. http://doi.org/10.1177/095624789901100120

United Nations. (2015). The Millennium Development Goals Report 2015. Retrieved from http://www.un.org/millenniumgoals/2015_MDG_Report/pdf/MDG%202015%20rev%20(July%201).pdf

United Nations. (n.d.). Goal 7 Fact Sheet: Millennium Development Goals and Beyond 2015. Retrieved from http://www.un.org/millenniumgoals/pdf/Goal_7_fs.pdf

WHO | Diarrhoeal disease. (n.d.). Retrieved February 24, 2016, from http://www.who.int/mediacentre/factsheets/fs330/en/

WHO/UNICEF. (2010). JMP Technical Task Force Meeting on Monitoring Drinking-water Quality. Villie-Morgon, France. Retrieved from http://www.wssinfo.org/fileadmin/user_upload/resources/JMP-Task-Force-Meeting-on-Monitoring-Drinking-water-Quality.pdf

WHO/UNICEF. (2015a). Ecuador: Estimates on the use of water sources and sanitation facilities.

WHO/UNICEF. (2015b). Honduras: Estimates on the use of water sources and sanitation facilities. Joint Monitoring Programme for Water Supply and Sanitation.

WHO/UNICEF. (2015c, June). Bolivia (Plurinational State of): Estimates on the use of water sources and sanitation facilities. Joint Monitoring Programme for Water Supply and Sanitation.

WHO/UNICEF. (n.d.). Definitions and Methods: wat/san categories. Retrieved February 3, 2016, from http://www.wssinfo.org/definitions-methods/watsan-categories/

WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation (JMP). (2011). Drinking Water Equity, Safey and Sustainability; Thematic report on drinking water.

Williams, A. R., Bain, R. E. S., Fisher, M. B., Cronk, R., Kelly, E. R., & Bartram, J. (2015). A Systematic Review and Meta-Analysis of Fecal Contamination and Inadequate Treatment of Packaged Water. PLoS ONE, 10(10), 1–27. http://doi.org/10.1371/journal.pone.0140899

World Health Organization (WHO). (n.d.). Guidelines for Drinking-water Quality, Fourth Edition. Retrieved February 24, 2016, from http://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf

Wutich, A., Beresford, M., & Carvajal, C. (2016). Can Informal Water Vendors Deliver on the Promise of A Human Right to Water? Results From Cochabamba, Bolivia. World Development, 79, 14–24. http://doi.org/10.1016/j.worlddev.2015.10.043

Chapter 3: Manuscript

An Analysis of the Microbial Quality of Packaged Water in Four sites in Latin America

Karla R. Feeser

3.1 Abstract

INTRODUCTION: Diarrheal disease contributes to an estimated 1.5 million deaths each year, including 760,000 deaths among children under the age of five. Of those, approximately 500,000 are attributable to inadequate drinking water. In areas where piped water is unsafe, unreliable or economically impossible, packaged water sold by private vendors can play an important role in meeting the water needs of these populations. As the activity and importance of packaged water vendors grow, more data is needed to assess the quality of water sold, and to inform policies that regulate the private water sector throughout the world.

AIM: This pilot study seeks to identify factors that may contribute to the deterioration of packaged water quality.

METHODS: Small packaged water enterprises (SWEs) operating in the following cities were visited twice between May 2014 and September 2015: La Paz, Bolivia; Tegucigalpa, Honduras; and Muisne and Tena, Ecuador. A brief survey was conducted with each distributor, and a facility tour was completed. Water samples were collected directly from the purification system and water packaged in both reusable and disposable containers were purchased. Samples were tested for total coliform and *E. coli* bacterial contamination on the day of collection and over the course of 28 days. Data was analyzed using descriptive statistics, including median as the measure of central tendency, and frequency where the main outcome was presence or absence of either total coliform bacteria or *E. coli*. To determine the factors that were most associated with water quality deterioration, logistic regression was performed.

RESULTS: A total of 616 samples were collected. This study found that 52% of the packaged water examined was contaminated with total coliform bacteria. Raw, untreated water and treated water packaged in reusable containers were most likely to be contaminated with total coliform bacteria and *E.coli* compared to treated water taken directly from the system. There was no significant association between water treatment or bottle disinfection protocols and total coliform or *E. coli* contamination.

DISCUSSION: The study succeeded in identifying at what stage and in what type of container water is most likely to be contaminated with bacterial water-quality indicators. Furthermore, it highlights the heterogeneity that exists in terms of types of water sold, water treatment systems, and sanitizing protocols among SWEs in Central and South America. Reusable containers are vulnerable to contamination with total coliform bacteria and *E. coli*, even when filled with clean water, thus the contamination may be due to inadequate disinfection between uses. These results may have implications for national or international policies that regulate private water enterprises, and can inform guidelines for packaged water distributors in particular. Further research is needed to identify optimal cleaning methods for reusable containers that are practical for use in lower resource settings.

3.2 Introduction

Globally, 780 million people lack access to improved drinking water (WHO & UNICEF, 2012). In developing regions, piped drinking water supplies reach only 46% of the population, due, in part, to disparities in access to safe water infrastructure. Piped water systems often do not reach lower income or rural areas in low-middle income countries where the established infrastructure required of a centralized water utility is often missing, and the high cost to develop missing infrastructure may be prohibitive (Dada, 2011). Where a centralized utility is available, it may be perceived as unsafe to drink (Hatt, 2006). Indeed, the microbial quality of piped drinking water sources is not globally monitored; they are thought to be clean due to the nature of their construction. If these sources are poorly maintained or constructed, they may be contaminated with fecal pathogens. Thus, it is likely that the number of people with access to a safe and improved source of drinking water is even less than estimates allow (WHO & UNICEF, 2012).

In Latin America, access to improved drinking water sources and piped water supplies in particular has risen substantially since 1990 to reach approximately 94% coverage, although substantial disparities between urban and rural areas still exist (WHO & UNICEF, 2012). However, throughout the region, there is mistrust in the quality of the water provided through centralized utilities (de Queiroz, Doria, 2013; Espinosa-García et al., 2015; Jain, 2014).

A growing number of people throughout the world are using bottled or packaged drinking water to fill gaps in water access and water quality. From 1990 to 2010, the number of people using bottled water rose from 37 million to 228 million and the number of people relying on private water venders more than doubled (WHO & UNICEF, 2012). Although exact data for Central and South America are limited, reports suggest that people throughout these regions are also increasingly dependent on private water enterprises for packaged, purified drinking water (Kjellen, 2006; Opryszko, 2009; Pacheco-Vega, 2015). Oftentimes, even where piped water is available, families may tolerate the added expense because they perceive the water as safe to drink, more so than other sources (Kjellen, 2006).

Water can be packaged into a variety of containers—disposable or reusable, bottles or sachets—and the delivery of water to consumers may vary greatly in different settings; however, reusable 20 liter (L) bottles are found in LMICs throughout Central and South America (Liu, 2013; Malkin, 2012). Typically, these plastic, cylindrical, narrow mouthed bottles are filled with water when purchased from a water distributor. When consumers empty the bottle, they bring it back to the distributor and the distributor either refills the bottle or exchanges the empty bottle for a full one. Despite their widespread use, little is known about the quality and safety of these large reusable bottles. In fact, despite many studies regarding the safe treatment of drinking water, there is a substantial gap in the scientific and public health literature concerning the quality of reusable 20L bottles and how to best implement and measure the impact of any associated interventions.

Companies that produce and sell bottled water in the United States are regulated by the U.S. Food and Drug Administration (FDA) and have standard protocols for inspecting and cleaning reusable containers (Nestle Waters North American Inc., 2010). However, despite the existence of international and national drinking water guidelines (World Health Organization, 2011), there is no available evidence to suggest that these kinds of stringent process for the disinfection of reusable containers are enforced in many low income countries throughout the world.

Poorer countries may face more obstacles in the monitoring and regulation of packaged water enterprises. In fact, even as private water distributors gain recognition as viable drinking water alternative, in many developing nations they operate either completely unregulated or unregistered (Dada, 2011). Consequently, studies investigating the microbial contamination of packaged water often find that the quality of packaged water sold by private distributors meets neither international guidelines nor national standards, where they are in place (Fisher et al., 2015; Halage et al., 2015).

This study seeks to characterize the quality of packaged water available for purchase in four sites in Central and South America: La Paz, Bolivia; Tegucigalpa, Honduras; Muisne, Ecuador; and Tena, Ecuador. Furthermore, it identifies factors that may contribute to the deterioration of packaged water quality, particularly in reusable containers, and discusses recommendations for the regulation of distributors that provided packaged water to underserved communities around the world.

3.3 Materials and Methods

3.3.1 Study Setting and Design

This study was carried out as a pilot project in four study sites across three Central and South American countries between May 2014 and September 2015. The study was undertaken by the non-profit organization Water Ecuador in the summer of 2015 in La Paz, Bolivia and in Tegucigalpa, Honduras with the collaboration of the Zamorano Escuela Agricola Panamericana. In Ecuador, the study was carried out in Muisne in between May and August 2014 and in Tena in between May and August 2015.

In each study site, packaged water distributors were identified using an informal reconnaissance survey to identify the most popular brands sold in local stores and directly from trucks or kiosks. Once identified, water distributors were visited during their normal operating hours so that researchers could collect samples, purchase water and interview the business manager.

This protocol was approved by the Georgia State University Institutional Review Board (IRB) and designated as not human subjects research (GSU IRB protocol number: H16339).

3.3.2 Company Surveys

A brief survey was conducted with each distributor at the Tegucigalpa, Tena and Muisne study sites to determine how many bottles were sold, how sales varied throughout the year, and where and how bottles were most often sold. The researcher also requested a tour of the facilities to view the treatment and cleaning procedure (Appendix 1)

3.3.3 Sample Collection

Five types of water samples were collected from small packaged water distributors during the study, depending on the consent of the business manager and on the availability of packaged water products for purchase. If available, raw water was collected from source water feeding the treatment system at a water distribution company or from a point along the system prior to any disinfection point. Treated water was sampled directly from the distributor's treatment system. Packaged water products were also sampled and these consisted of: sachets, disposable bottles or reusable containers. Sachets are small, sealed plastic pouches filled with water. These are intended for single-use and cannot be filled with water again. Disposable bottles were typically available in slightly larger volumes ranging from 500mL to 4L. They were made of a weak plastic, intended for one-time use. The most abundant container types were 20L reusable bottles. They are sold by the water distribution companies, and can be returned and refilled or exchanged for full bottles.

Tegucigalpa. In Honduras, eight unique distributors were visited on three occasions with a one-week time interval between visits. At each visit, one sample of crude water and one sample of treated water were collected, and three 20L reusable bottles of water were purchased. Crude and treated water samples were tested on the day of the visit, along with one of the three 20L bottles. The remaining two bottles were tested on day 7 and day 31 following the visit.

La Paz. In La Bolivia, eight unique distributors were visited twice each, with a minimum of one week in between visits. Samples were not obtained directly from the purification systems but 20L reusable bottles were purchased from each distributor and sachet water was purchased from six of eight distributors.

Muisne. In Muisne, treated water samples were collected, and both small, disposable (4L) bottles and large, reusable (20L) bottles were purchased. Six unique distributors were visited. Four were visited twice each, and two were visited once. At the first visit, researchers conducted an interview with a company representative, collected two samples of treated water directly from the distribution system, and purchased four small (4L) disposable bottles and twelve large (20L) reusable bottles that had been filled with water that same day. Researchers returned to each distributor a minimum of one week later to collect two additional samples of treated water, and purchase 16 more disposable and reusable bottles—four and twelve, respectively. Disposable and reusable bottles were inspected upon purchase and stored, unopened, away from direct sunlight, in the laboratory where temperature varied from 23.5 – 27^0 C, until testing. Treated water samples were labeled on site, and tested within 3 hours on Day 0. 20L reusable bottles were tested in duplicate 0, 3, 7, 14, 21 and 28 days from the date of collection. Non-reusable bottles were tested 0, 7, 21, and 28 days from the date of collection (Figure 1).

Tena. Six unique distributors were visited in Tena. Five were visited twice each, and one was visited one-time only. Sample collection and distributor visits were conducted in Tena as in Muisne with the following change: instead of twelve, ten large (20L) reusable bottles were purchased at each distributor and tested in duplicate on Days 0, 3, 7, 14 and 21.

3.3.4 Testing Procedures

In accordance with the World Health Organization Guidelines for Drinking-water Quality, *E. coli* and total coliforms were used as indicators of fecal contamination and inadequate water treatment, respectively (WHO, 2011). Bottles and sachets were visually inspected for damage, and the water assessed for turbidity and evident contamination, such as mold or insects. A 100mL sample of water from each bottle or sachet was collected and tested per manufacturer's instructions (IDEXX Laboratories, 2015). Briefly, one packet of IDEXX reagent was added to the sample. The sample-reagent mixture was poured into a sterile Quantitray 2000. Trays were sealed using a small iron. Care was taken to ensure that the hot iron did not touch any part of the tray for longer than 10 seconds, to avoid overheating. Samples were incubated at 35±0.5°C for

24 hours. Total coliform and *E. coli* concentrations were determined using the corresponding IDEXX Most Probable Number (MPN) table (IDEXX Laboratories, 2013).

Two negative controls were undertaken for each experiment: one with a 500mL Dasani-brand bottled water, which was available for purchase at all study sites, and one sample of distilled medical-grade water, purchased from a pharmacy. A positive control containing a mixture of sewer and borehole water was performed at the end of each experiment to avoid contaminating testing equipment.

3.3.5 Data Analysis

The data for each sample were entered in Microsoft Excel and imported into SAS 9.4 for further analysis. Data was analyzed using descriptive statistics, including median as the measure of central tendency, and frequency where the main outcome was presence or absence of either total coliform bacteria or *E. coli*. Data was observed for trends in frequency and severity of contamination in relation to storage time. Logistic regression was performed to compare the proportion of treated water contaminated with total coliform or *E. coli* to the proportion of other sample types that were contaminated.

Restricting the analysis to reusable containers, logistic regression was used to compare the proportion of bottles contaminated in Tena (where the highest prevalence of contamination existed) to the proportion of contaminated bottles in other study sites. To determine the effect of the number of interactions between researchers and distributors (i.e. whether repeat visits to the same manufacturer influenced the cleanliness of the samples), logistic regression was used to compare the proportion of contaminated bottles collected on the third visit to the proportion of contaminated bottles collected on the first visit. Where company survey data was available, each company was classified into exclusive categories for water treatment methods and for bottle cleaning methods. These variables were analyzed as above to determine their associations with total coliform and *E. coli* contamination. Multivariate logistic regression analysis was performed on all categorical variables to produce adjusted odds ratios for total coliform contamination in reusable containers.

3.4 Results

3.4.1 Survey Results

Surveys were completed at a total of 20 distributors with eight from Tegucigalpa, Honduras, and six each from Muisne and Tena in Ecuador. No surveys or tours were completed during visits to distributors in La Paz. All used carbon activated filtration in their water treatment systems. All but one (95%) used ultraviolet (UV) sterilization, and most companies who used UV in their water treatment systems also used ozone sterilization (75%). Sand filtration was used by all companies in Tegucigalpa, by 66% in Muisne, and by one company in Tena (17%). Microfiltration was used less frequently in water distribution systems of the companies surveyed (35%). Only one company (5%), based in Tena, used chlorine in its water treatment system.

Ninety-five percent of companies (n=19) indicated some procedure for cleaning reusable bottles. Approximately seventy percent used hot water—either mechanized or manually—to clean bottles and 65% used some kind of detergent. Fifteen percent used only hot water to sanitize bottles. One used only a cold water rinse. Less than a quarter of companies washed bottles with a chlorine solution or used ozone to sterilize reusable bottles (Table 1).

Table 1. Overview of the 20 companies surveyed in three of four study sites (Tegucigalpa, Muisne and Tena).

Site	Distributor	Water Treatment Method[1]	Method for Cleaning Containers[2]
Tegucigalpa	A	SF + UV	CW + Detergent
Tegucigalpa	B	SF + MF + UV	CW + Detergent
Tegucigalpa	C	SF + UV	HW only
Tegucigalpa	D	SF only	HW + Detergent
Tegucigalpa	E	SF + MF + UV + Chem	None
Tegucigalpa	F	SF + MF + UV + Chem	HW + Detergent
Tegucigalpa	G	SF + MF + UV + Chem	HW + Detergent
Tegucigalpa	H	SF + MF + UV + Chem	HW + Detergent
Muisne	A	SF + MF + UV + Chem	None
Muisne	B	MF + UV + Chem	HW + Det. + Chlorine
Muisne	C	UV + Chem	HW + Detergent
Muisne	D	SF + MF + UV + Chem	HW + Det. + Chlorine
Muisne	E	SF + MF + UV + Chem	HW + Detergent
Muisne	F	SF + MF + UV + Chem	HW + Detergent
Tena	A	MF + UV + Chem	None
Tena	B	MF + UV	HW + Det. + Chlorine
Tena	C	MF + UV + Chem	CW + Detergent
Tena	D	UV + Chem	HW only
Tena	E	UV + Chem	HW only
Tena	F	SF + UV + Chem	CW only

[1]All distributors surveyed used Carbon Activated Filters to treat water; SF = Sand Filtration, UV = Ultraviolet Irradiation, Chem = Chemical disinfection with Chlorine or Ozone.

[2] CW= Cold Water, HW = Hot Water

3.4.2 Detection of Total Coliforms and *E. coli* in water samples

A total of 616 samples were collected or purchased from a total of 28 distributors across the four study sites (Table 2). Overall, the median concentration of total coliform bacteria was 51.9 cfu/100mL (range: <1 – 200.5) in raw water, 19.2 cfu/100mL (range: <1 - >2419.6) in water packaged in reusable bottles, 19.05 cfu/100mL (range: <1 -211.0) in water packaged in disposable bottles, <1 cfu/100mL (max: >2419.6) in sachet water, and <1 cfu/100mL (max: >2419.6) in treated water samples. The median for E. coli was <1 cfu/100ml of all types of samples. The median *E. coli* concentration was 0.0 cfu/100mL in raw water (max: 200.5), water packaged in reusable bottles (max: 472.1), water packaged in disposable bottles (max: 0.0), and in treated water samples (max: 154.2) (Table 3).

Table 2. Water Sample Type by Study Site

Site (No. of Distributors)	Raw (%)	Treated (%)	Disposable Bottle (%)	Sachet (%)	Reusable Bottle (%)	Total
Tegucigalpa (8)	24 (20.0)	24 (20.0)	0	0	72 (60.0)	120
La Paz (8)	0	0	0	110 (67.9)	52 (32.1)	162
Muisne (6)	0	22 (11.8)	36 (19.4)	0	128 (68.8)	186
Tena (6)	0	22 (14.9)	16 (10.8)	0	110 (17.9)	148
Total	24 (3.9)	68 (11.0)	52 (8.4)	110 (17.9)	362 (58.8)	616

In Tegucigalpa, the median level of total coliform contamination was 0.0. cfu/100mL (max: 25.4) among treated water samples, and 8.7 cfu/mL (range: 0 – 200.5) among samples taken from reusable containers. No E. coli was detected in treated water samples collected in Tegucigalpa. The median E. coli concentration among samples taken from reusable bottles was 0.0 cfu/100mL (max: 9.9).

Table 3. Descriptive statistics of total coliform and *E. coli* concentration in each sample type.

Parameter	Median[1]	IQR	Max.	Min.
Raw Water (n=24)				
Total coliform	51.9	[159.5 – 12.7]	200.5	<1
E. coli	<1	[0.5 – <1]	65.9	<1
Treated Water (n= 68)				
Total coliform	<1	[2.6 – <1]	2419.6	<1
E. coli	<1	0.0	154.2	<1
Disposable Bottle (n=52)				
Total coliform	<1	[5.8 – 0.0]	211	<1
E. coli	<1	NA	<1	<1
Sachet Water (n=110)				
Total coliform	<1	0.0	2419.6	<1
E. coli	<1	0.0	<1	<1
Reusable Bottle (n=362)				
Total coliform	19.2	[203.7 – <1]	2419.6	<1
E. coli	<1	0.0	472.1	<1

[1]Total coliform bacteria and *E. coli* concentrations are present in units of CFU per 100ml

In Muisne, the median total coliform concentration was <1 cfu/100mL in treated water samples (max: 7.5) and in samples taken from disposable bottles (max: 178.2); it was 23.1 cfu/100mL among samples taken from reusable bottles (range: 0 – >2419.6). No *E. coli* was detected in treated water samples or in samples from disposables bottles collected in Muisne. The median *E. coli* contamination in samples taken from reusable bottles was 0.0 cfu/100mL (max: 24.9).

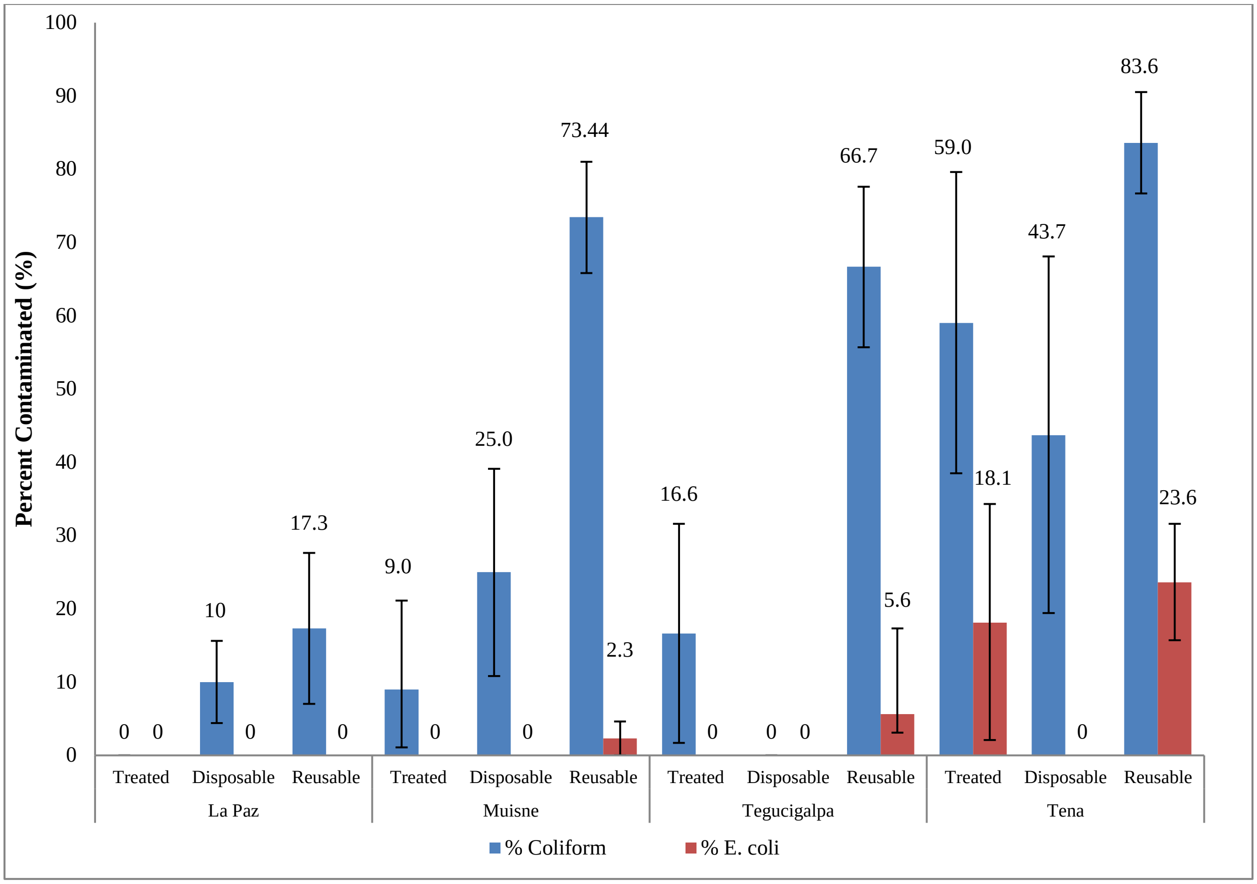
In Tena, the median total coliform concentration was 13.2 cfu/100mL in treated water samples (max: >2419.6) and 0.0 cfu/100mL among reusable bottle samples (max: 108.9); it was 121.3 cfu/100mL among samples taken from non-reusable bottles (max: >2419.6). The median E. coli contamination was 0.0 cfu/100mL in treated water samples (max: 154.2), and in samples taken from reusable containers (max: 472.1). No *E. coli* were detected in samples taken from disposable bottles. In La Paz, the median level of coliform contamination was 0.0 cfu/100mL among samples taken from reusable containers (max: >2419.6).

Total coliform bacteria were detected in 311 (50.5%) water samples. Total coliform bacteria was most frequently detected in raw water (22/24; 91.7%) and in samples taken from reusable bottles (243/362; 67.1%). Sachet and treated water were contaminated with total coliform bacteria least frequently (10.0% and 27.9%, respectively) (Figure 2).*E. coli* contamination was found in 45 (7.3%) water samples. *E. coli* was most frequently found in raw water samples (6/24; 25.0%) and in samples taken from reusable bottles (35/362; 9.7%). No *E. coli* were detected in samples taken from non-reusable containers, such as disposable bottles (0/52) or sachet water (0/110) (Figure 2). Ten of twenty companies sampled at the point of treatment were found to have contamination with total coliforms even after treatment. All samples from reusable bottles that were purchased from these companies were contaminated with total coliforms as well. Of the ten companies whose treated water was free from total coliform contamination, nine samples of water from reusable bottles were contaminated with total coliforms (Table 4). No noticeable degradation in water quality was observed between samples tested on the day of the visit and those tested after being stored for up to 28 days (Table 5).

Table 4. Associations of company-treated water and coliform contamination

Quality of Reusable Bottles Sold	**Total Coliform Contamination in Treated Water (%)**	**No Total Coliform Contamination Detected in Treated Water (%)**	**Total**
Total Coliform Contamination Detected	10 (35.7)	9 (53.6)	19
No Contamination Detected	0 (0.0)	1 (10.7)	1
Total	10	10	20

Figure 2. Percent of samples contaminated with Total Coliforms and *E. coli* by site and type



[a] Non-reusable water sources include sachet water and disposable bottles.
[b] La Paz-No data for treated water available
[c] Tegucigalpa-No data for reusable bottles available
*Error bars represent 95% Confidence Intervals

3.4.3 Results of Analyses

Association between sample type and Total Coliform or E. coli contamination

Raw, untreated water and water packaged in reusable bottles were significantly more likely to be contaminated with total coliforms than treated water taken directly from purification systems (ORs= 28.4 and 5.3, respectively). Sachet water was least likely to be contaminated with total coliform bacteria (OR = 0.3 compared to treated water) (Table 6).

Table 5. Quality of water by post-vist time sampling time point and sample type

Sample Type	Sampling Time Point	n	Total Coliform Detected (%)	*E. coli* Detected (%)
Treated	0	68	19 (27.9)	4 (5.8)
Sachet	0	52	7 (13.4)	0 (0)
	3	28	3 (10.7)	0 (0)
	7	30	1 (3.3)	0 (0)
Disposable Bottles	0	13	4 (30.7)	0 (0)
	7	13	3 (23.0)	0 (0)
	14	4	1 (25.0)	0 (0)
	21	13	4 (30.7)	0 (0)
	28	9	3 (33.3)	0 (0)
Reusable	0	120	58 (48.3)	10 (8.3)
	3	44	36 (81.8)	6 (13.6)
	7	68	58 (85.2)	7 (10.2)
	14	44	33 (75.0)	7 (15.9)
	21	44	36 (81.8)	4 (9.0)
	28	42	22 (52.3)	1 (5.0)

[1]Post-visit sampling time point is in indication of the number of days since the sample was purchased or collected from the distributer. Time-point 0 indicates that the sample was tested on the same day as purchased.

No *E. coli* were detected in any samples taken from disposable bottles or sachet water. Raw, untreated water was most likely to be contaminated with *E. coli* (OR = 5.3 compared to treated water). *E coli* contamination was found more frequently in reusable bottles than in treated water, but the resulting OR (OR= 1.7) was not statistically significant (95% CI: 0.6 – 5.0) (Table 7).

Factors Associated with Coliform contamination of Reusable Bottles in Univariate Analyses

When restricting univariate analyses to samples taken from reusable bottles (n=362) as these were the only sample type collected in all study sites and from all distributors, study site was significantly associated with total coliform contamination (p<0.01). Water packaged in reusable containers was most likely to be contaminated with total coliforms in Tena and least likely to be contaminated in La Paz (OR= 0.04 [0.0- 0.1]).

Table 6. Association between sample type and prevalence of total coliform contamination

	Total Coliform			
Sample Type	**Present**	**Absent**	**Total**	**OR (95% CI)**
Raw Water	22 (91.7)	2 (8.3)	24 (100)	28.4 (6.1 -132.5)
Treated Water	19 (27.9)	49 (72.1)	68 (100)	*ref.*
Disposable Bottle	16 (30.8)	36 (69.2)	52 (100)	1.1 (0.5 - 2.5)
Sachet Water	11 (10.0)	99 (90.0)	110 (100)	0.3 (0.1 - 0.7)
Reusable Bottle	243 (67.1)	119 (32.9)	362 (100)	5.3 (2.9- 9.3)
Total	311 (50.5)	305 (49.5)	616 (100)	

The treatment methods used in the water distribution system were not significantly associated with total coliform contamination of reusable bottles, but bottle cleaning methods were. Water sampled from reusable bottles that were purchased from distributors who used only hot water to clean bottles were more likely to be contaminated with total coliforms than those that were cleaned with a combination hot water, detergent and chlorine bleach (OR=6.3 [1.9 – 19.9]. Total coliform contamination was found in all reusable bottles that were cleaned with only cold water (OR= >999.9), but due to this complete separation of data the finding is not significant (Table 8). When samples taken from reusable bottles purchased from distributors whose treated water samples tested positive for contamination were removed from analyses, no bottle cleaning methods remained significant (p=0.4).

Factors Associated with E.coli contamination of Reusable Bottles in Univariate Analyses

In univariate analyses, water treated using carbon filtration, UV irradiation and chemical disinfection was significantly more likely to be contaminated with *E. coli* than water treated with these methods plus sand and membrane filtration (OR= 16.3[4.6 – 57.9]). Similar to total coliform contamination, bottles cleaned with HW only were significantly more likely to be contaminated with *E. coli* than those cleaned with HW, detergent and chlorine (OR= 10.3 [2.8 – 37.7]) (Table 9). When samples taken from reusable bottles purchased from distributors whose treated water samples tested positive for contamination were removed from analyses, no bottle cleaning methods remained significant (p=0.7).

Table 7. Association between sample type and prevalence of *E. coli* contamination.

		E. coli		
Sample Type	**Present**	**Absent**	**Total**	**OR (95% CI)**
Raw Water	6 (25.0)	18 (75.0)	24 (100)	5.3 (1.4 - 20.9)
Treated Water	4 (5.9)	64 (94.1)	68 (100)	*ref.*
Reusable Bottle	35 (9.7)	327 (90.3)	362 (100)	1.7 (0.6 - 5.0)

No *E. coli* was found in any samples collected from disposable bottles or sachet water.

Results of Multivariate Analyses

There was no survey data available for companies visited in La Paz, so the multivariate model was restricted to reusable bottles purchased in Tegucigalpa, Tena, and Muisne. Tena remained the most likely site for contamination of total coliform contamination, compared to Tegucigalpa which was the least likely to be contaminated (aOR = 0.03 [0.0 – 0.5]). No other factors remained significant in multivariate analysis (Table 8). Multivariate analysis was not conducted for *E. coli* contamination due to the low prevalence of *E. coli* across study sites which contributed to a questionable validity of the multivariate model.

Table 8. Results of Univariate and Multivariate Analyses for Presence of Total Coliform Bacteria Among Reusable Bottles

Exposure	No Total Coliforms Detected (%)	Total Coliforms Detected (%)	OR [95% CI]	*p*	aOR[1] [95%CI]	*p*
Site						
Tegucigalpa	24 (33.3)	48 (19.75)	0.4 [0.2 - 0.8]	0.3	0.0[0.0 - 0.5]	**<0.01**
La Paz	43 (82.7)	9 (17.3)	0.0 [0.0 - 0.1]	<0.01	--	--
Muisne	34 (26.6)	94 (73.4)	0.5 [0.3 -0.9]	<0.01	0.3[0.0 - 1.5]	0.1
Tena	18 (16.4)	92 (83.6)	*ref.*	*ref.*	*ref.*	*ref.*
Site Visit						
1st Visit	52 (28.6)	97(71.4)	1.3 [0.5 - 3.1]	0.3	1.5[0.5 - 4.6]	0.1
2nd Visit	59 (37.8)	97 (62.2)	0.8 [0.3 - 2.0]	0.3	0.7[0.2 - 2.2]	0.1
3rd Visit	8 (33.3)	16 (66.7)	*ref.*	*ref.*	*ref.*	*ref.*
Bottle Damage (n=17)	5 (29.4)	12 (70.6)	1.2 [0.4 - 3.4]	1	0.6 0.1 - 2.8]	0.5
Bottle Cleaning[1]						
None	20 (37.7)	33 (62.3)	0.9 [0.4 - 2.0]	0.8	0.6[0.2 - 1.9]	0.4
CW only[2]	0 (0.0)	10 (100.0)	--	--	--	--
HW only	4 (8.2)	45 (91.8)	6.3[1.9 - 19.9]	<0.01	4.1[0.3-54.4]	0.3
CW + Detergent	5 (13.2)	33 (86.8)	3.7[1.2 - 10.9]	0.02	2.3[0.3-21.2]	0.5
HW + Detergent	27 (26.0)	77 (74.0)	1.6 [0.8 - 3.2]	0.2	2.4 [0.8 -7.3]	0.1
HW + Detergent + Chlorine	20 (35.7)	36 (64.3)	*ref.*	*ref.*	*ref.*	*ref.*
Water Treatment[1]						
SF	3 (33.3)	6 (66.7)	0.8[0.2- 3.5]	*0.8*	1.2[0.3 - 5.8]	0.8
SF + UV	4 (22.2)	14 (77.8)	1.5 [0.5 - 4.8]	0.5	1.4[0.2-12.9]	0.8
MF + UV	9 (45.0)	11(55.0)	0.5 [0.2 - 1.3]	0.2	0.1[0.0 - 1.3]	0.08
UV + Chem	10 (15.6)	54 (84.4)	2.3 [1.1 - 4.9]	0.03	0.3[0.1 - 1.0]	0.06
SF + MF + UV	1 (11.1)	8 (88.9)	3.4[0.4 - 27.9]	0.3	3.6[0.2 - 67.9]	0.4
MF + UV + Chem	11(21.2)	41 (78.9)	1.6 [0.7 - 3.4]	0.2	0.4 [0.1 - 1.8]	0.3
SF + UV + Chem[2]	0 (0.0)	10 (100.0)	--	--	--	--
SF + MF + UV + Chem	38 (29.7)	90 (70.3)	*ref.*	*ref.*	*ref.*	*ref.*

[1]No surveys were conducted at water companies located in La Paz; reusable bottles collected and sampled in La Paz are excluded from these analyses.
[2]All bottles cleaned with CWO were filled with SF + UV + Chem treated water. These ten bottles were collected from a single distributor and all were contaminated with total coliform bacteria.

3.5 Discussion

In this pilot study, we were able to describe and identify substantial differences across 20 packaged water distributors in 4 sites in Central and South America. The quality of packaged water varied significantly between reusable and disposable containers, and between reusable containers purchased in different study sites. More than half of the packaged water examined in this study was contaminated with total coliform bacteria. Twenty liter reusable bottles were available for purchase at every distributor in all study sites, illustrating their ubiquity. The majority of reusable containers were contaminated with total coliform contamination, and *E. coli* was detected in the bottles sold in three of the four study sites.

Table 9. Results of Univariate Analyses for Presence of *E. coli* Among Reusable Bottles

Exposure	No *E. coli* Detected (%)	*E. coli* Detected (%)	OR [95% CI]	*p*
Site[1]				
Honduras	68 (94.4)	4 (5.6)	0.2 [0.1 - 0.6]	0.9
La Paz	52 (100)	0 (0)	---	---
Muisne	125 (97.7)	3 (2.3)	0.1 [0.0 - 0.3]	0.9
Tena	84 (76.4)	26 (23.6)	*ref.*	*ref.*
Site Visit				
1st Visit	164 (90.1)	18 (9.9)	1.2 [0.3 -5.6]	0.7
2nd Visit	143 (91.7)	13 (8.3)	1 [0.2 -4.7]	0.9
3rd Visit	22 (91.7)	2 (8.3)	*ref.*	*ref.*
Bottle Damage (n=17)	16 (94.1)	1 (5.9)	0.6 [0.1 - 4.8]	1
Bottle Cleaning[1]				
None	52 (98.1)	1 (1.9)	0.3 [0.0 - 3.4]	0.4
CW only	8 (80)	2 (20)	4.4 [0.6 - 30.6]	0.1
HW only	31 (63.2)	18 (36.7)	10.3 [2.8 - 37.7]	<0.01
CW + Detergent	32 (84.2)	6 (15.8)	3.3 [0.8 - 14.2]	0.1
HW + Detergent	101 (97.1)	3 (2.8)	0.5 [0.1 - 2.7]	0.4
HW + Detergent + Chlorine	53 (94.7)	3 (5.4)	*ref.*	*ref.*
Water Treatment[1]				
SF	8 (88.9)	1 (11.1)	5.2 [0.5 - 55.9]	0.2
SF + UV	17 (94.4)	1 (5.6)	2.5 [0.2 - 24.9]	0.4
MF + UV	19 (95.0)	1(5.0)	2.2 [0.2 - 22.2]	0.5
UV + Chem	46 (14.9)	18 (5.9)	16.3 [4.6 - 57.9]	<0.01
SF + MF + UV	8 (88.9)	1 (11.1)	5.2 [0.5 -55.9]	0.2
MF + UV + Chem	46 (88.5)	6 (11.5)	5.4 [1.3 - 22.6]	0.02
SF + UV + Chem	8 (80.0)	2 (20.0)	10.4 [1.5 -71.5]	0.02
SF + MF + UV + Chem	125 (97.7)	3 (2.3)	*ref.*	*ref.*

Multivariate analyses were not conducted for *E. coli* due to the low prevalence of *E. coli* contamination across study sites and sample types which contributed the questionable validity of the multivariate model.
[1]No E. coli was detected in any sample collected in La Paz and no surveys were conducted at water companies located in this study site; reusable bottles collected and sampled in La Paz are excluded from these analyses.

The treated water samples tested from half of distributors were free from total coliform contamination (n= 10, 50%). Yet of the ten companies whose treated water samples were free from total coliform contamination, nine (90%) of them sold bottles that were positive for total coliform contamination. Based on these observations, it is likely that the primary source of contamination found in water packaged in reusable bottles stems from the bottles themselves, and may be due to inadequate disinfection between uses. The present study found no link between particular bottle cleaning methods and water contamination; although there is some evidence to suggest that the addition of chlorine and detergent to hot water cleaning protocols can prevent contamination. However, data collection assessed bottle cleaning method by a single self-report survey item that did not evaluate adherence to reported cleaning protocols, so it remains unclear whether adherence to these protocols is consistent. It is recommended in future research to collected data on bottle cleaning methods using several survey items that will evaluate adherence to protocols as well.

Total coliforms are indicator organisms, and their presence in treated water can be used to determine if water treatment processes were adequate to destroy bacterial pathogens. It is possible for water containing total coliform bacteria to be free of human pathogens; however, it is unlikely that a water sample would contain pathogenic bacteria and test negative for total coliform bacteria. *E .coli* are also indicator organisms, and can be used to determine whether drinking water has been exposed to a source of fecal contamination such that there could be pathogenic organisms present. The drinking water guidelines set forth by WHO dictate that there should be no detectable E. coli per 100mL of water intended for drinking, including packaged or bottled water (WHO, 2011). Despite the added expense and level of trust that consumers have, the water bottled in these study sites demonstrated coliform and E. coli contamination levels that may pose a substantial health risk to consumer (Bain, 2014). These results may have implications for national or international policies that regulate private water enterprises, and can inform guidelines for packaged water distributors in particular.

The elaborate bottle cleaning techniques used by large bottled water providers that operate in higher income countries may not be feasible for the small distributors that operated in the studied settings. Even when the appropriate technologies are in places, failure in protocol adherence can occur. For example, companies the operate in the U.S. clean their large reusable containers vigorously, and reuse them a certain number of times-- up to 35-- before recycling them (Nestle Waters North American Inc., 2010). In resource poor settings, bottles may be reused more times than manufacturer recommendations in order to save money and resources.

3.6 Limitations of the Research

This study has several limitations. While the study was conducted in four distinct geographical regions in attempt to gain a more representative sample of LMICs in Central and South America, the heterogeneity of packaged water available for purchase in each study site limited the bulk of the analyses to reusable 20L containers. Furthermore, the quality of water differed significantly between companies within each site. Therefore, little can be said regarding how or if the quality of packaged water varies between regions. Additionally, the study was conducted during a single time of year, and samples were collected from only a fraction of the private bottled water enterprises that operate throughout these regions. Finally, the study does not account for the different kinds of storage and transportation conditions that can occur between bottling and point of use.

Manuscript References

Bain, R., Cronk, R., Hossain, R., Bonjour, S., Onda, K., Wright, J., … Bartram, J. (2014). Global assessment of exposure to faecal contamination through drinking water based on a systematic review. Tropical Medicine & International Health, 19(8), 917–927. http://doi.org/10.1111/tmi.12334

Dada, A. (2011). Packaged water: optimizing local processes for sustainable water delivery in developing nations. Globalization & Health, 7(1), 24–31. http://doi.org/10.1186/1744-8603-7-24

Datamonitor. (2004). Bottled Water Industry Profile: Latin America (p. 1). MarketLine, a Progressive Digital Media business. Retrieved from http://ezproxy.gsu.edu/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=bth&AN=27275369&site=eds-live

de Queiroz, J. T. M., Doria, M. de F., Rosenberg, M. W., Heller, L., & Zhouri, A. (2013). Perceptions of bottled water consumers in three Brazilian municipalities. Journal of Water and Health, 11(3), 520–531. http://doi.org/10.2166/wh.2013.222

Espinosa-García, A. C., Díaz-Ávalos, C., González-Villarreal, F. J., Val-Segura, R., Malvaez-Orozco, V., & Mazari-Hiriart, M. (2015). Drinking water quality in a Mexico city university community: perception and preferences. EcoHealth, 12(1), 88–97. http://doi.org/10.1007/s10393-014-0978-z

Falcone-Dias, M. F., Emerick, G. L., & Farache-Filho, A. (2012). Mineral water: a microbiological approach. Water Science & Technology: Water Supply, 12(5), 556–562. http://doi.org/10.2166/ws.2012028

Fisher, M. B., Williams, A. R., Jalloh, M. F., Saquee, G., Bain, R. E. S., & Bartram, J. K. (2015).

Microbiological and Chemical Quality of Packaged Sachet Water and Household Stored Drinking Water in Freetown, Sierra Leone. PLOS ONE, 10(7), e0131772. http://doi.org/10.1371/journal.pone.0131772

Halage, A. A., Ssemugabo, C., Ssemwanga, D. K., Musoke, D., Mugambe, R. K., Guwatudde, D., & Ssempebwa, J. C. (2015). Bacteriological and physical quality of locally packaged drinking water in Kampala, Uganda. Journal of Environmental and Public Health, 2015, 942928. http://doi.org/10.1155/2015/942928

Hatt, L. E., & Waters, H. R. (2006). Determinants of child morbidity in Latin America: A pooled analysis of interactions between parental education and economic status. Social Science & Medicine, 62(2), 375–386. http://doi.org/10.1016/j.socscimed.2005.06.007

IDEXX Laboratories. (2013). Quanti-Tray/2000 Insert and Most Probable Number (MPN) Table. Retrieved from https://www.idexx.com/resource-library/water/quanti-tray-2000-procedure-en.pdf

IDEXX Laboratories. (2015). Colilert. Retrieved from https://www.idexx.com/resource-library/water/colilert-procedure-en.pdf

Impellitteri, C. (2007). US EPA Small Drinking Water Systems: State of the Industry and Treatment Technologies to Meet the Safe Drinking Water Act Requirements September 2007 - lps100419.pdf. Retrieved April 2, 2016, from http://permanent.access.gpo.gov/lps100419/lps100419.pdf

Informe anual bebidas 2015. (2015). Industria Alimenticia, 26(8), 12–19.

Jain, M., Lim, Y., Arce-Nazario, J. A., & Uriarte, M. (2014). Perceptional and socio-demographic factors associated with household drinking water management strategies in rural Puerto Rico. PloS One, 9(2), e88059. http://doi.org/10.1371/journal.pone.0088059

J. Liu, N. d'Ozouville. (2013). Water contamination in Puerto Ayora: Applied interdisciplinary research using Escherichia coli as an indicator bacteria. Galapagos Report 2011-2012, 76–83.

Kjellen, M., & McGranahan, G. (2006). Informal Water Vendors and the Urban Poor. International Institute for Environment and Development (IIED). Retrieved from http://pubs.iied.org/pdfs/10529IIED.pdf

Madeline Baer. (2015). From Water Wars to Water Rights: Implementing the Human Right to Water in Bolivia. Journal of Human Rights, 14(3), 353–376. http://doi.org/10.1080/14754835.2014.988782

Malkin, E. (2012, July 16). Mexicans Struggle to Kick Bottled-Water Habit. The New York Times. Retrieved from http://www.nytimes.com/2012/07/17/world/americas/mexicans-struggle-to-kick-bottled-water-habit.html

Marzano, M. a., Ripamonti, B., & Balzaretti, C. m. (2011). Short communication: Monitoring the bacteriological quality of Italian bottled spring water from dispensers. Food Control, 22, 333–336. http://doi.org/10.1016/j.foodcont.2010.06.014

Nestle Waters North American Inc. (2010). Poland Spring Water Quality Report. Stamford: Nestle Waters.

Opryszko, M. C., Huang, H., Soderlund, K., & Schwab, K. J. (2009). Data gaps in evidence-based research on small water enterprises in developing countries. Journal of Water and Health, 7(4), 609–622. http://doi.org/10.2166/wh.2009.213

Pacheco-Vega, R. (2015). Agua embotellada en México: de la privatización del suministro a la mercantilización de los recursos hídricos. Bottled Water in Mexico: From Privatization of Supply to Marketization of Water Resources., 22(63), 221–263.

WHO & UNICEF. (2012). Progress on Drinking Water and Sanitation: 2012 Update. Joint Monitoring Programme for Water Supply and Sanitation. Retrieved from http://www.unicef.org/media/files/JMPreport2012.pdf

Williams, A. R., Bain, R. E. S., Fisher, M. B., Cronk, R., Kelly, E. R., & Bartram, J. (2015). A Systematic Review and Meta-Analysis of Fecal Contamination and Inadequate Treatment of Packaged Water. PLoS ONE, 10(10), 1–27. http://doi.org/10.1371/journal.pone.0140899

World Health Organization. (2011). Guidelines for Drinking Water Quality, Fourth Edition. WHO. Retrieved from http://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf

Chapter 4: Conclusion

A key finding from this study is that water packaged in reusable containers is significantly more likely to be contaminated than water packaged in other containers, including disposable bottles and sachets. Furthermore, the contamination likely stems from inadequate cleaning of the bottles between uses. This finding is supported by a small number of studies comparing large 20 liter reusable containers to other packaged water types in the body of literature on packaged water quality (Falcone-Dias, 2012; Marzano, 2013; Levesque, 1994). Previous studies have reported on the quality of sachet water, and have found it to be poor—perhaps owing to the fact that sachet water is more of an informal industry and perhaps less often scrutinized by regulatory frameworks (Dada, 2011; Stoler, 2012). This study somewhat contradicts those findings. No fecal contamination was found in any of the sachet water samples, but sachet water was only available for purchase in a single study site, so this discrepancy cannot be addressed with the current data available.

This study reports on packaged water quality in two rural areas in Ecuador, which contributes to the body of literature on packaged water quality which contains few studies conducted in rural areas (Williams et al., 2015). Furthermore, this study highlights the heterogeneity that exists in terms of types of water sold, water treatment systems, and sanitizing protocols among SWEs in Central and South America.

Reusable containers are vulnerable to contamination with total coliform bacteria and *E. coli*, even when filled with clean water. These results may have implications for national or international policies that regulate private water enterprises, and can inform guidelines for packaged water distributors in particular. For regulating bodies to ignore packaged water's role in filling gaps in access to improved drinking water systems is to allow unregulated and—and perhaps unaware—private enterprises to distributed contaminated water. Further research is needed to identify optimal cleaning methods for reusable containers that are practical for use in lower resource settings.

5. Appendices

APPENDIX A: Survey questions administered to the business managers at each bottled water distributer.

WATER ECUADOR

Survey Questions (Spanish)

Información básica

¿Cuándo su empresa comenzará a funcionar?
¿Cuántos bidones se venden por semana?
¿Cómo varían la ventas según la temporada?
¿Dónde venden botellas?
¿Cuánto venden sus jarras de agua 20L para que los consumidores / empresas?

Información de la empresa

Hábleme de cómo opera su empresa (por ejemplo, obtiene y purifica el agua). (Nota para el topógrafo - esta pregunta es para minimizar el sesgo de composición abierta.)
Si necesita, use estas sondas: Fuente de agua, proceso de tratamiento (RO, ozono, UV, carbón activado, filtros de sedimentos, cloro, sulfato de aluminio, de reducción de la dureza, etc.) proceso de distribución, proceso de saneamiento jarra, proceso de contratación, el número de empleados
¿Cómo ha cambiado la demanda de agua embotellada en los últimos 5-10 años?
¿Cómo cree que la demanda de agua embotellada va a cambiar en el futuro?

Información interactiva

¿Puedo tener un tour?
Nota prácticas de higiene.
¿Puedo tener una muestra de su agua purificada directamente de su sistema?